SYNOPSIS OF P. G. RESEARCH PROBLEM

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4.	**Name of the Degree Submitted for**	:	Ph.D. (Agri.)
5.	**Major subject**	:	Genetics and Plant Breeding
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8.	**Title of research problem**	:	**MORPHO - BIOCHEMICAL AND MOLECULAR CHARCATERIZATION, CROSSABILITY STUDIES AND WIDE HYBRIDIZATION IN OKRA (*Abelmoschus* spp.) FOR VIRUS RESISTANCE AND YIELD IMPROVEMENT**

9. Introduction

Okra (*Abelmoschus esculentus* L. Moench) previously called as *Hibiscus esculentus* is a commercially important summer vegetable crop cultivated in tropical and sub-tropical areas in the world. Okra is also known as lady finger or *Bhindi* and is one of the major vegetables grown in the summer and rainy seasons in India. It belongs to the Malvaceae family and is characterized by its typical showy flower. Almost 99% of okra cultivation is distributed exclusively across the various developing countries of Asia and Africa (Singh *et al.,* 2023). The okra production in India is estimated to be around 7611.71 thousand tonnes from 561.22 thousand ha area of land and productivity of 13.56 Mt/ha out of which Gujarat contributes to a production of 1168.87 thousand tonnes from 93.96 thousand hectare of land with a productivity of 12.44 Mt/ha (INDIASTAT, 2024). Okra is a highly nutritious vegetable rich in essential vitamins, minerals, fiber, and antioxidants. It is an excellent source of vitamin C, vitamin A,

folate, and vitamin B complex, which are important for immune function, skin health, and metabolic processes (Liu *et al.,* 2021). It have chromosome number 2n=130 and a genome size of about 1.19 Gb (Wang *et al.,* 2023). Breeders and geneticists find okra to be an intriguing crop due to its monadelphous situation, large flowers that can be emasculated easily, and high seed production in a single pollination. Okra is classified as an often cross-pollinated crop with outcrossing of 4 to 30%.

Due to its high yield and short cropping cycle, okra is prominently grown in tropical and sub-tropical regions of the world, attracting investors and novice farmers (Kumar *et al.,* 2020). The Food and Agriculture organisation of the United Nations reports that okra output has significantly increased worldwide. India is the top producer, followed by Nigeria, Sudan, Mali, Pakistan, Côte d'Ivoire, Cameroon and Ghana (Cobbinah and Kwoseh, 2021). Africa is the geographical origin of okra (National Horticulture Board, 2017). Due to its large share of fresh vegetable exports, okra has enormous potential for foreign exchange earnings (APEDA, 2020). Okra's tender pods are utilized as a wonderful vegetable. Particularly in West Africa, its leaves, buds, and flowers are often eaten. It is somewhat utilized in frozen, tinned, and dehydrated forms. Okra mucilage is utilized in culinary, nonfood, and pharmaceutical products. Dry okra seeds are high in protein (20–23%) and oil (18–20%). Okra is important for nutrition since it contains vitamins and minerals like calcium and potassium, and its roots and stems are used to clean the cane juice during the sugar production process. While okra is eaten in salads, stews, and soups throughout the world, it is boiled, roasted, and fried in India (Salameh, 2014).

The two main biotic stresses in bhindi are Okra Enation Leaf Curl Disease (OELCuD) and Okra Yellow Vein Mosaic Disease (OYVMD), which are both brought on by viruses in the genus Begomovirus from the family Geminiviridae (Venkataravanappa *et al.,* 2016). The vector whitefly (*Bemisia tabaci*) is responsible for spreading these viruses. It is not economically viable to treat these viral diseases using chemical and mechanical means (Khade *et al.,* 2020; Santhiya *et al.,* 2022). Therefore, in order to generate resistant cultivars, it is esssential to identify and combine resistant sources. Due to the lack of a consistent resistance source for YVMV and OELCuD in cultivated *A. esculentus*, the hunt for a stable resistance source has switched to wild *Abelmoschus* species.

Various biochemical parameters govern the susceptibility or resistance of a genotype clearly revealed that the population of whiteflies were high in germplasm lines containing high total and reducing sugars and vice versa (Manju *et al*., 2021). Given that sugars are regarded as one of the essential nutrients for plants, the variation in the proportion of sugars between germplasm lines with varying susceptibilities to whiteflies suggested that these substances might function as stimulants to whiteflies feeding on okra. Thimmaiah *et al*. (1992) reported that resistant genotypes to whiteflies contain decreased amount of polysaccharides in all the tissues when compared to the susceptible genotypes in cotton .

Gangopadhyay *et al.* (2017), Suma *et al.* (2023) reported wild *A. manihot* subsp. manihot, *A. manihot* subsp. tetraphyllus, *A. caillei*, *A. moschatus* to be resistant to OYVMD. *A. angulosus* and *A. ficulneus* have resisitance against OELCuD (Singh *et al.* 2009). *A. moschatus* (IC 141055) and *A. manihot* var. tetraphyllus (IC 90476-1) are resistant to both OELCuD and OYVMD (Santhiya *et al.,* 2022 and Badiger *et al.,* 2024). Interspecific crossings and subsequent selection among segregating generations can be used to access these important wild genetic resources (Suma *et al.,* 2023; Badiger *et al.,* 2024). Pre-breeding lines with resistance features found in wild species are eventually produced as a result of this process.

10. Practical Utility

The insect vector that naturally spreads viruses is the whitefly (*Bemisia tabaci* Genn.), one of the most destructive insects that harms crops by carrying the OELCV and YVMV in addition to directly causing plant vitality loss through feeding on cell sap. In domesticated species, there is little genetic diversity and only a few sources of resistance available, which is the main lacunae in overcoming this disease (Nagaraju *et al.,* 2019). Here comes the need of exploring wild relatives of okra as novel source of resistance. Thakur and Arora (1986) identified *Abelmoschus angulosus, A. tetraphyllus, A. moschatus, A. caillie* and *A. manihot* as viable and steady resistance source to several diseases. Researchers have specifically used *A. manihot, A. manihot* sp. manihot, and *A. tetraphyllus* to introduce yellow vein mosaic virus (YVMV) resistance into cultivated okra. Since wild relatives have resistant genes and have been shown to be devoid of OELCV and YVMV, they are an essential source of resistance (Singh *et al*., 2009).

The selection of stable and heritable resistance sources that can be advanced in breeding pipelines is made easier by characterisation. Additionally, it makes it possible to create molecular markers associated with resistance, which speeds up marker-assisted selection (MAS). Furthermore, screening in controlled and field settings aids in separating escape mechanisms from real resistance, guaranteeing selection dependability. Additionally, research on these populations sheds light on linkage drag, gene interactions, and the possibility of combining resistance to ELCV and YVMV in a single genetic background. This is especially useful for creating robust, broad-spectrum resistant cultivars.

Breeders can find appropriate parental combinations for successful hybrid development by using crossability analysis, which offers crucial insights into the compatibility relationships between cultivated and wild species. The development of successful strategies to resolve pre and post-fertilization obstacles, such as pollen–pistil incompatibility, pollen tube growth inhibition, hybrid embryo abortion, and sterility, is made possible by using strategies which include the use of methods like embryo rescue, bridge crosses, and chromosome doubling. Crossability evaluation also helps determine the direction of viable crosses (direct or reciprocal).

Screening makes it possible to accurately identify resistant donors and ideal cross combinations early, shortening the breeding cycle. Screening F_1 hybrids offers insights into the manifestation of resistance and the nature of gene action, evaluation of parental lines guarantees the selection of genetically stable and dependable sources of resistance. Comparative evaluation of parents and hybrids also makes it easier to identify heterosis for virus resistance.

Reliable sources of resistance offer a solid genetic base for breeding initiatives, guaranteeing consistent performance in a range of settings and disease pressure levels. This is particularly crucial for vector-borne viruses, since environmental changes can affect the frequency and severity of disease. By lowering the chance of resistance breakdown and requiring fewer screening cycles, using reliable resistance donors improves breeding efficiency. It makes it possible for breeders to more precisely and confidently introduce well-characterized resistance genes into elite genetic backgrounds. Additionally, the production of cultivars with long-lasting and broad-

spectrum resistance is made easier by the use of stable resistance sources, which helps to prevent the establishment of new virulent pathogen strains. Advanced breeding techniques like gene pyramiding, which combines several resistant genes from several sources to provide long-lasting protection, are also supported by the selection of trustworthy donors. Furthermore, these sources provide useful genetic material marker creation and molecular characterisation, which speeds up marker-assisted selection. The development of high-yielding, virus-resistant okra cultivars or obtaining a line which is resistant to virus that can lessen reliance on chemical vector control, minimize production costs, and support economically and environmentally viable agriculture, is the ultimate practical utility of the proposed research project.

11. Objectives

Keeping this in view, the present investigation on "Morpho-Biochemical and Molecular Charcaterization, Crossability studies and Wide Hybridization in okra (*Abelmoschus* spp.) for Virus Resistance and Yield Improvement" will be undertaken with the objectives listed below:

1. Morpho-Biochemical characterization of parents for virus resistance and diversity.
2. Molecular characterization of parents for genetic diversity.
3. To study the crossability behaviour of parents and success of wide hybridisation.
4. Evaluation and identification of F_1 for virus resistance and yield parameters.

12. Review of Literature

The literature pertaining to the characterization, crossability behaviour, F_1 and F_2 screening for disease resistance and other horticultural traits in okra has been reviewed under the following subheadings.

12.1 Morpho-Biochemical Characterization

Mishra *et al.* (2015) examined 33 okra germplasm for the relative contribution of thirteen quantitative traits to genetic divergence. The study found that yield contributing variables, particularly the number of diseased plants (29.92%) and yield per plant (26.89%), contributed most to genetic divergence. Fruiting time (13.45%), fruit weight (8.14%), days to first harvest (7.95%), days to 50% flowering (6.06%), fruit girth (2.84%), first flowering node (1.14%), fruit length and number of fruits per

plant(0.76%), and plant height (0.19%) were additional characteristics that contributed to divergence.

Chandramouli *et al.* (2016) examined the relative contributions of various traits to overall genetic divergence. According to the study, the percentage of YVMV infestation (42.53%) and leaf area (31.72%) contributed the most to overall divergence, followed by fruit yield per plant (14.25%), fruit yield per plot (9.20%), days to 50% flowering (1.15%), fruit weight (0.92%), and first flowering node (0.23%). They have recommended choosing different parents in order to take advantage of heterosis in okra.

Gangopadhyay *et al.* (2017) evaluated 68 accessions of four wild *Abelmoschus* species, *Abelmoschus caillei* (A. Chev.) Stevels, *Abelmoschus manihot* (L.) Medik, *Abelmoschus moschatus* (L.) Medik, and *Abelmoschus tuberculatus* as well as eight cultivated okra varieties were characterized for biotic stress-related and phenological traits under natural epiphytotic conditions. According to their research, *Abelmoschus* species exhibit a comparatively high degree of variety in phenological traits and traits linked to biotic stressors. PCA, or principal component analysis, aided in the precise separation of species with the least amount of overlap..

Silva *et al.* (2021) evaluated 46 Brazilian type okra accessions, and the results showed that there was genetic variability among the accessions. Based on the multivariate Ward-MLM procedure, these 46 genotypes were divided into seven groups; the characteristics that contributed most to the diversity were leaf width, leaf length, petiole length, basal stem diameter, and plant height at first flowering.

Oppong-Sekyere *et al.* (2020) investigated 25 okra accessions that were from Ghana and assessed them for morphological characteristics such as phenotypic diversity and quality; a dendrogram was created using these morphological data grouped 25 genotypes into four clusters. This study revealed that distinct morpho-types were present in Ghanaian okra germplasms; these characteristics include fruit shape, anthocyanin pigmentation, leaf and stem pubescence, and petal color.

Temam *et al.* (2020) conducted an assessment program using 36 genotypes of okra.With the exception of leaf breadth, leaf length, and the number of epicalyx, the analysis of variance of morphological characteristics revealed a considerable degree of variation between the genotypes.

12.2 Evaluation of extent of genetic and molecular diversity in Okra

Kyriakopoulou *et al.* (2014) employed AFLP markers to investigate the genetic diversity and origin of okra genotypes. These markers displayed a clear pattern of geographic grouping and barely 12% polymorphism. Greek germplasms exhibited a unique clustering pattern with a considerable degree of heterogeneity in pod and seed characteristics among the germplasms employed.

Yuan *et al*. (2014) examined the genetic diversity and differentiation of twenty-four okra genotypes using ISSR markers. Out of it, 22 ISSR primers produced 289 amplified DNA fragments, of which 145 (or 50%) were polymorphic. A dendrogram was created using unweighted pair-group with arithmetic average (UPGMA) clustering based on the outcomes of these 289 ISSR markers. Twenty-four okra genotypes were divided into four geographically separate groups by Dendrogram. This analysis's average PIC (Polymorphic Information Content) was 0.531.

Kumar *et al.* (2017) assessed the genetic diversity of 96 *Abelmoschus* accessions, comprising 92 genotypes of *A. esculentus* and one genotype each from *A. moschatus* ssp. tuberosus, *A. moschatus*, *A. tuberculatus*, and *A. manihot* using 40 SSR primers. Only 30 of these 40 SSRs have generated repeatable bands, comprising 213 bands. The polymorphism information content was 0.52 on average.

Sandeep *et al*. (2025) examined 38 genotypes of okra (*Abelmoschus esculentus*) for genetic diversity, and their potential for hybrid breeding through single, double, and multi-parent crossings was assessed. The cultivars were divided into ten clusters according to morphological diversity based on important phenotypic characteristics, demonstrating significant genetic variation. Using 35 polymorphic microsatellite markers (SSR) to measure molecular diversity, 91 alleles were found, classifying the cultivars into ten genetically distinct clusters

12.3 Crossability

Prabu and Warade (2013) carried out crossability research on okra (*Abelmoschus*) cultivars and found that while *A. ficulneus* and *A. esculentus* were compatible, their seeds were sterile when *A. esculentus* was used as the female parent, whereas the cross is incompatible when *A. ficulneus* is employed as the female parent. *A. esculentus* was compatible with wild relatives such as *A. manihot* spp. manihot, *A. manihot* spp. tetraphyllus, *A. tuberculatus*, and *A. manihot* (L.) in both direct and

reciprocal crosses. Both Medikus and *A.caillei* are incompatible with *A. moschatus* in both ways.

Reddy (2015) reported a successful cross, as evidenced by hybrid lethality and interspecific F1 free from hybrid breakdown, although the hybrids were entirely sterile. *A. esculentus* (RNOYR-19) is the female parent used in the crossing program, whereas *A. manihot* subsp tetraphyllus is the male parent. Fertility has been partially restored in interspecific F1 plants treated with 0.1% colchicine on their apical meristems.

Amiteye *et al.* (2019) evaluated both intraspecific and interspecific hybridization between and within species, ten accessions of *Abelmoschus* spp., including two species*, A. esculentus* (T1, T2, T3, VT, ID, and AG) and *A. callei* (KB, AM, YL, and T4), collected from six different geographical regions of Ghana, were crossed in every possible combination. With a high degree of crossability index (CI) (45.71% to 90.32%), all six accessions of *Abelmoschus esculentus* were able to hybridize with one another in both direct and reciprocal cross combinations. However, when *A. esculentus* was utilized as females, cross-compatibility between *A. callei* and *A. esculentus* was only successful in one direction, with a CI between 34.48% and 60%. The most compatible female and male parental lines were found to be T3 and T1.

Nagaraju *et al.* (2019) reported that YVMV and ELCV viruses provide significant obstacles to okra farming, necessitating the use of wild-related taxa to include resistance. However, hybrid development and utilization is difficult because of F1 hybrid sterility issues. They have confronted issues like the inability to advance the interspecific hybrids to next generation because of lack of viable seed during selfing and backcrossing the advanced lines in crosses using *A. esculentus* with *A. angulosus* var. grandifloras, *A. tuberculatus*, *A. tetraphyllus* and *A. mizonagensis* sp. (Mizoram). They reported that fruit and seed sets in a hybrid between *A. caillei* and *A. esculentus* were good, ranging from 66.7 to 100%. However, poor seed germination results from a shortage of viable embryos, which could be caused by an imbalance in endosperm numbers.

Singh and Pathak (2020) found the percentage of fruit set in direct crosses of A. esculentus cultivar Anakomban x *A. manihot* & *A. esculentus* cultivar Anakomban x *A. manihot* spp tetraphyllus. When compared to the reciprocal cross, the fruit set percentage was low; this difference suggests partial incompatibility. The sluggish development of the pollen tube was the cause of this partial incompatibility. The low seed set percentage caused the seeds to shrivel, primarily as a result of inadequate

endosperm development. Four hybrids were pollen sterile as a result of meiotic irregularity caused by differences in chromosomal numbers between the species involved in the crossing. In hybrids, pollen sterility and seed set percentage are adversely correlated. Endosperm degeneration may be the cause of certain seeds that look normal but are empty.

Sandeep *et al.* (2022) carried out crossability investigations in three wild species of okra—*A. ficulneus*, *A. manihot*, and *A. moschatus*—as well as the cultivated okra variety Arka Anamika were used in. Only when *A. esculentus* was utilized as a seed parent were the crosses successful. Lower germination in *A. moschatus* and *A. ficulneus* was caused by a hard seed coat. Crosses of Arka Anamika × *A. moschatus* and Arka Anamika × *Abelmoschus ficulens* showed reduced seed germination. On the other hand, 92% of the seeds germinated in the Arka Anamika × *A. manihot* hybrid. Additionally, morphological characterization of interspecific hybrids showed that these hybrids tended more toward wild parents and were erect and abundantly growing as compared to parental species.

Suma *et al.* (2023) identified tolerant wild *Abelmoschus* species(*A. pungens* var. mizoramensis, *A. enbeepeegearense*, *A. caillei*, *A. tetraphyllus*, and *A. angulosus* var. grandiflorus) through germplasm screening at hotspots. These species were used in wide-hybridization programs to generate interspecific hybrids, introducing desirable genes with some linkage drag. Most wide cross derivatives exhibited intermediate fruit morphology and dominant wild characters (hispid fruits, stem, leaves, tough fiber, vigorous growth, prolonged flowering/fruiting). However, three BC progenies showed high morphological similarity to cultivated okra, confirming successful transfer of useful genes to the cultivated okra gene pool

Singh *et al.* (2024) evaluated crosses between cultivated okra and kasturi okra to understand their compatibility. Direct crosses (*A. esculentus* × *A. moschatus*) showed good fruit set, seed formation, and germination, while reciprocal crosses failed due to poor seed development caused by embryo degeneration. However, even successful hybrids were sterile and produced seedless fruits, indicating hybrid sterility. The study suggests that although crossing is possible, fertility needs to be restored (e.g., using colchicine) before these hybrids can be used in breeding programs to transfer resistance traits.

Santhiya *et al.* (2025) conducted a hybridisation study considering *Pusa A-4* and different wild species to evaluate compatibility and resistance. Among them, *A.*

ficulneus showed better cross compatibility, but the strongest resistance to YVMV and OELCuD was observed in hybrids derived from *A. moschatus*. Although initial hybridization was challenging, a successful backcross population was developed using *A. moschatus*-derived resistant plants. These backcross plants largely regained cultivated characteristics while retaining high levels of disease resistance. Molecular marker analysis further confirmed their hybrid nature and closer similarity to the cultivated parent.Overall, the results highlight *A. moschatus* as an excellent donor for viral disease resistance and demonstrate that such interspecific hybrids can be effectively used in breeding programs to develop improved, disease-resistant okra varieties.

12.3 Evaluation and identification of F1

Kumari *et* al. (2021) carried out field screening of wild okra *(Abelmoschus moschatus* ssp. moschatus) accessions for OELCuD resistance. Out of 76 wild okra accessions tested, 10 accessions, viz. EC360586, EC360794, EC360830, EC360900, EC359730, EC359836, EC359870, EC360351, EC361111 and EC361171 showed resistant (R) reaction in Kharif 2017, whereas in Kharif 2019, only four accessions, viz. EC360794, EC360586, EC360830 and EC361171 exhibited R reaction and remaining six accessions were moderately resistant (MR). In the first year, average percent disease index (PDI) was 14.15 and overall PDI ranged from 3.70 to 52.86. The range of PDI was 4.53–56.40 during the second year with an average PDI value 18.04

Kanthaswamy *et* al. (2021) evaluated nineteen okra hybrids for resistance to OSFB, OELCV and Powdery Mildew Disease (PMD), using commercial CO 4 as check. The findings indicated that OKHYB-4 had a very low YVMV incidence (2.60%). Anna-10 had a reduced incidence of OSFB (9.11% PDI). Interestingly, none of the hybrids showed signs of OELCV or PMD. According to these results, OKHYB-4 and Anna-10 are suggested for commercial cultivation in the Karaikal coastal region, subject to testing particular to the area and time of year.

Santhiya *et* al. (2022) assessed the OYVMD and OELCuD resistance of 24 wild okra genotypes. High resistance was demonstrated by five genotypes (*A. moschatus* IC 141055, *A. tetraphyllus* IC 90476-1, *A. caillei* (Sikkim), *A. moschatus* EC 360900, *A. tetraphyllus* IC 90515/IC 470735), which are appropriate for interspecific breeding and resistant variety generation.

Santhiya *et al.* (2025) crossed four wild accessions with the cultivated okra variety *Abelmoschus esculentus* cv. 'Pusa A-4', *A. moschatus* (IC-141055), *A. manihot* var. tetraphyllus (IC 90476-1), *A. caillei* (Sikkim), and *A. ficulneus* (Sel-1). *A. ficulneus* (Sel-1) was found to be more compatible with cultivated okra than other wild relatives in cross-compatibility tests. In comparison to their interspecific F1 hybrids and BC_1F_1s, both cultivated and wild parents had limited variability in pollen diameter and high pollen viability. Under natural conditions, interspecific F1 hybrids were screened for okra yellow vein mosaic disease (OYVMD) and okra enation leaf curl disease (OELCuD). The results showed that while interspecific lines derived from *A. ficulneus* were susceptible, those derived from *A. moschatus* were highly resistant.

Roy *et al*. (2026) examined the morphological, biochemical, and disease resistance characteristics of 16 different okra genotypes and 21 F_1 hybrids from ahalf-diallel mating scheme over three growing seasons. Field phenotyping is used with fuzzy C-means clustering and regression modeling to predict the beginning of disease and categorize genotypes along the course of disease development. The accuracy of genotype selection is increased by the fuzzy classification method's successful capture of intermediate resistance reactions. Genotypes with decreased symptom expression, particularly YVMV, are found using regression analysis, and their promise in resistance breeding is emphasized.

13. Materials and Methods

13.1	Location	:	Vegetable Research Farm, College of Agriculture, J.A.U, Junagadh
13.2	Year and season of parent screening and crossing	:	*Kharif* 2026
13.3	Number of parents used for initial viral screening		6 to 10 based on availability
13.4	Parents utilized for crossing programme		As per the virus screening results (approx 4 to 6)
13.5	Year and season Screening of f_1 and parents		Summer 2027
13.6	Design		Randomized Block Design

13.7	Number of replications		3
13.8	Crossing pattern	:	Crosses will be made in all possible combinations
13.9	Spacing	:	75 x 75 cm

13.10 List of genotypes used for crossing

Sr. No.	Genotype
1	*Abelmoschus esculentus*
2	*Hibiscus sabdariffa*
3	*Abelmoschus moschatus*
4	*Abelmoschus manihot*
	Genotype 1
	Genotype 2

- Other species like *Abelmoschus angulosus, A. tuberculatus, A. crinitus, A. palianus, A. caillei, A. ficulneus, A. enbeepeegearense, A. rugosus, A. rhodopetallus, A. palianus* may be utilized in the research work depending upon the availability of the germplasm.

13.11 Observations to be recorded

Morphological characters will be observed. From each replication, five plants will be chosen at random to record observations. The list of observations is provided below:

13.11.1 Plant height (cm)

Plant height of the random selected plants will be recorded in (cm) from ground level to tip at maturity and then averaged .

13.11.2 No. of branches per plant

It will be recorded by counting the effective branches per plant at full maturity stage and then averaged.

13.11.3 First flowering node (cm)

The node at which first fruit appears will be recorded from the selected five plants and then averaged.

13.11.4 Numbers of nodes per plant

The total number of nodes on the main stem of plant will be recorded and then averaged.

13.11.5 Internodal length (cm)

Total number of fruits obtained from randomly selected plants in each entry during each picking of marketable fruits will be recorded as number of fruits per plant.

13.11.6 Fruit length (cm)

Mature fruits from each randomly selected plants will be selected during the peak period of harvesting for recording the fruit length. The length in centimeter will be recorded from the basal tip of fruit and then averaged to find the length of fruit.

13.11.7 Fruit yield/plant (g)

Fruit yield per plant, measured in grams, will be determined by averaging the fruit weight from all of the periodic harvests in five randomly chosen plants of each genotype.

13.11.8 Average fruit weight (g)

During each harvesting of marketable fruits, the number of fruits will be taken from randomly chosen plants will be noted by weighing them in grams, and the average fruit weight will be calculated.

13.11.9 No. of days and time of Anthesis

The no. of days from sowing to the opening of first flower will be recorded from the randomly selected five plants and then averaged.

13.11.10 Leaf characters

13.11.10.1 Leaf length (cm)

Five randomly chosen plants will be used for leaf lengths measurement. Three fully opened leaves will be chosen from a plant, and length of each leaf will be measured using a scale. The average leaf length will be calculated centimeters.

13.11.10.2 Leaf area (cm^2)

Five randomly chosen plants will be used for leaf area measurement for each germplasm. A leaf area meter will be used to measure the area of three fully opened leaves from a plant and then averaged.

13.11.11 Flower Characters

13.11.11.1 Flower colour

The colour of flower will be recorded visually and noted down.

13.11.11.2 Flower diameter

The diameter of three fully opened flowers from the randomly selected plants will be measured using a measuring scale in cm and then averaged.

13.11.11.3 Stylar length

The length of the style will be measured in cm using a measuring scale starting from the filament till the top of ovary.

13.11.11.4 Flower longevity

Time period in hours will be recorded between anthesis to petal closure from randomly selected flowers.

13.11.12 Trichome Characters

13.11.12.1 Trichome density estimation (nos./mm^2)

Three uniformly developed leaves will be chosen at random from each germplasm. A binocular microscope with a 10x magnification will be used to examine the leaf fragments. Trichomes in a 1 cm^2 leaf region will be counted, and the average will be computed.

13.11.12.2 Trichome Length

Ocular micrometer will be used for measuring the trichome length.

13.11.13 Histochemical staining

Leaves will be plucked from each respective genotype and then washed with double distilled water then subjected to histochemical Diaminobenzidine (DAB) and Nitrobue tetrazolium (NBT) staining. Then it will be destained to observe the accumulation of H_2O_2 and $O_2^{\bullet-}$ to observe the injury caused due to the virus infection.

13.11.14 Crossability indices

In order to calculate the crossability index and hybrid lethality, data on the number of crosses attempted, self-pollinated, fruits set (%), seed set/fruit, F1 seed germination (%), and % F1 plant set fruits with or without seeds will be recorded. The formula provided by (Rao *et al.*, 1981) will be used to determine the crossability index (%).

13.11.14.1 Fruit set (%)

$$\text{Fruit set (\%)} = \frac{\text{No. of fruits set}}{\text{Total no. of flowers pollinated}} \text{ X } 100$$

13.11.14.2 Average no. of seeds per fruit

$$\text{no. of seeds per fruit} = \frac{\text{Total no. of seeds obtained}}{\text{No. of fruits set}} \text{ X } 100$$

13.11.14.3 Germination percentage (%)

$$\text{Germination percentage} = \frac{\text{Seeds germinated}}{\text{Total seeds sown}} \text{ X } 100$$

13.11.14.4 Hybrid lethality

$$\text{Hybrid lethality} = \frac{\text{No. of plants died}}{\text{Total no. of seeds germinated}} \text{ X } 100$$

13.11.15 Pollen viability

1% acetocarmine staining will be used to evaluate the pollen viability of parental species and their F_1s.

$$\text{Viable pollen grain (\%)} = \frac{\text{No. of stained pollen grains}}{\text{Total no. of pollen grains}} \text{ X } 100$$

13.11.16 Biochemical analysis

13.11.16.1 Estimation of total phenols

The total phenol content will be estimated as per Bray and Thorpe (1954).

13.11.16.2 Estimation of Stress related enzymes

Stress related enzymes like Peroxidase, Polyphenol oxidase and Esterase will be done using method described by Prabodhbhai P. K. (2019).

13.11.16.3Estimation of Total sugars

13.11.17 Assesment of disease severity and its classification

The okra OELCV and YVMV disease prevalence will be documented based on the disease symptoms generated on different wild okra lines and damage score (0->4 scale) will be utilized for assessing the per cent disease incidence (Venkataravanappa *et al.*, 2022).

Scale for categorizing Okra's response to OELCV and YVMV disease

Symptoms	Severity	Response value	Reaction
Symptoms absent	0	0	HR
Very mild symptoms upto 25% leaf area	1	0.25	R
Appearance of disease between 26-50% leaf area	2	0.50	MR
Symptoms between 51-75% leaf area	3	0.75	MS
Severe disease infection at 75% leaf area	4	1.0	S
Above 75% leaf area	>4	>1.00	HS

HR: Highy resistant, R: Resistant, MR: Medium Resistant, MS: Medium susceptible, S: Susceptible, HS: Highly susceptible

$$\text{Percent Disease Infection (\%)} = \frac{\text{infected plant}}{\text{total no. of plant}} \text{ X } 100$$

The Response Value (RV) assigned for the severity grade value and the PDI of YVMV & OELCV are multiplied to determine the coefficient of infection (CI).

$$\text{CI (\%)} = \text{PDI x RV}$$

14. Statistical Analysis

For each character, the mean values of the parents and F_1 hybrids will be subjected to the following statistical analysis :

14.1 Analysis of Variance (ANOVA)

The obtained morphological data from *Abelmoschus* genotypes will be subjected to Analysis of variance studies as per Panse and Sukhatme (1985).

14.2 Coefficient of variance (C.V.)

The coefficient of variation (CV) will be calculated by using the following formula

$$\text{C. V. (\%)} = \frac{\text{Standard Deviation}}{\text{Mean}} \text{ X } 100$$

14.3 Heterosis estimation

(A) Heterobetiosis

$$\text{Heterobeltiosis (\%)} = \frac{\bar{F}_1 - \overline{BP}}{\overline{BP}} \times 100$$

Where,

$\bar{F}_1$ = Mean performance of F_1

$\overline{BP}$ = Mean value of better parent of respective cross combination

(B) Standard Heterosis

$$\text{Standard heterosis (\%)} = \frac{\bar{F}_1 - \overline{SC}}{\overline{SC}} \times 100$$

Where,

$\bar{F}_1$ = Mean performance of F_1

$\overline{SC}$ = Mean performance of the standard check commercial variety

14.4 Molecular Diversity

Molecular diversity will be assessed using SSR markers

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